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Chronic mitragynine (kratom) enhances punishment resistance in natural reward seeking and impairs place learning in mice

Nurul Iman W. Ismail[1,2], Nanthini Jayabalan[1,2], Sharif Mahsufi Mansor[3], Christian P. Müller[4] & Mustapha Muzaimi[1,2]

Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Malaysia[1], Centre for Neuroscience Services and Research, Universiti Sains Malaysia, Malaysia[2], Centre for Drug Research, Universiti Sains Malaysia, Malaysia[3] and Section of Addiction Medicine, Department of Psychiatry and Psychotherapy, Friedrich-Alexander-University Erlangen-Nuremberg, Germany[4]

ABSTRACT

Kratom (*Mitragyna speciosa*) is a widely abused herbal drug preparation in Southeast Asia. It is often consumed as a substitute for heroin, but imposing itself unknown harms and addictive burdens. Mitragynine is the major psychostimulant constituent of kratom that has recently been reported to induce morphine-like behavioural and cognitive effects in rodents. The effects of chronic consumption on non-drug related behaviours are still unclear. In the present study, we investigated the effects of chronic mitragynine treatment on spontaneous activity, reward-related behaviour and cognition in mice in an IntelliCage® system, and compared them with those of morphine and Δ-9-tetrahydrocannabinol (THC). We found that chronic mitragynine treatment significantly potentiated horizontal exploratory activity. It enhanced spontaneous sucrose preference and also its persistence when the preference had aversive consequences. Furthermore, mitragynine impaired place learning and its reversal. Thereby, mitragynine effects closely resembled that of morphine and THC sensitisation. These findings suggest that chronic mitragynine exposure enhances spontaneous locomotor activity and the preference for natural rewards, but impairs learning and memory. These findings confirm pleiotropic effects of mitragynine (kratom) on human lifestyle, but may also support the recognition of the drug's harm potential.

Keywords kratom, mitragynine, morphine, place learning, sucrose preference, Δ-9-tetrahydrocannabinol (Δ9-THC).

Correspondence to: Mustapha Muzaimi, Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. E-mail: drchik@hotmail.com/mmuzaimi@usm.my

INTRODUCTION

The recent proliferation of unregulated psychoactive and recreational substances is unprecedented in the chronicles of drug addiction, further imposing public health implications worldwide (Goldberg 2014). Different classes of psychoactive substances share common behavioural and neurobiological patterns, despite their diverse pharmacological profiles and resultant adverse effects (Edwards & Koob 2013; Koob 2011). Recent understandings in the neurobiological mechanism of addiction have been derived from extensive studies on animal models of addictions on widely popular drugs of abuse, such as opioids, cannabinoids, stimulants, nicotine and alcohol (Edwards & Koob 2013; Goldberg 2014; Koob 2011; Müller & Homberg 2015). In this study, mitragynine was primarily tested due to its widespread use in human, despite unknown cognitive long-term effects.

In Malaysia, the National Anti-Drugs Agency (NADA 2013) has declared a sudden rise in the raid and seizures of novel synthetic drugs, such as ecstasy and psychotropic pills, as well as a local psychoactive plant, kratom (better known as ketum in Malaysia). There is a growing trend among local drug addicts to use the leaves of

Malaysian folklore medicinal plant, kratom (*Mitragyna speciosa* Korth) as opioid replacement (Hassan *et al.* 2013; Jansen & Prast 1988; Sakaran *et al.* 2014; Suwanlert 1975) and to ameliorate withdrawal to other drugs (Ahmad & Aziz 2012; Singh, Müller, & Vicknasingam 2014; Vicknasingam *et al.* 2010). Herbal preparations of kratom are increasingly found and used in Europe and the US, either as pure preparation (Cornara et al., 2013; Forrester, 2013) or as one herbal ingredient of 'legal-' or 'herbal high' preparations, which are distributed under various names such as *Krypton, K2 or Spice* (Dresen et al., 2010; Arndt et al., 2011). The upsurge of kratom abuse has raised considerable concern due to its morphine-like behavioural and neurological effects, hence subjects to high addictive liabilities (Ahmad & Aziz 2012; Saingam *et al.* 2013; Suwanlert 1975). Consequently, Malaysian authorities had recently announced that this addictive plant will be gazetted under the Dangerous Drugs Act of 1952 for a tougher and stringent control measure.

The main psychoactive alkaloid of kratom is mitragynine, as well as its more potent but less abundant congener, 7-hydroxymitragynine (Hassan *et al.* 2013; Sakaran *et al.* 2014; Takayama 2004). Extensive studies of mitragynine demonstrated the high binding affinity for μ-, δ- and κ-opioid receptors governing their antinociceptive and antitussive actions (Shamima *et al.* 2012; Takayama 2004; Thongpradichote *et al.* 1998). A single dose of 20 mg/kg of mitragynine resulted in an increase of horizontal and vertical exploratory activities in novel Y-maze in mice (Hazim, Mustapha, & Mansor 2011), while 10, 20 and 40 mg/kg acute administration of mitragynine increased central zone and open arms exploration in open field and plus maze tests (Hazim *et al.* 2014). Acute administration of kratom standardised methanol extract in high dosage significantly improved learning acquisition, but impaired long-term memory consolidation in rats in passive and active avoidance tests (Senik *et al.* 2012). Long-term exposition to mitragynine, however, may have numerous adverse effects. A study by Sabetghadam *et al.* (2013) demonstrated neuropathological changes in hippocampal white matter, frontal lobe, medulla and cerebellum of rats after chronic treatment with high doses of mitragynine. Significant reduction of open-field locomotor activity and impaired working memory was observed in mice (Apryani *et al.* 2010), and acquisition, consolidation, as well as retrieval of passive avoidance learning was independently impaired after chronic mitragynine administration in rats (Yusoff *et al.* 2014). However, a multi-dimensional characterization of chronic mitragynine effects is still lacking. We performed a longitudinal study using IntelliCage® learning system, which allowed an extended behavioural and cognitive analysis of socially interacting mice exposed to chronic mitragynine. We compared the behavioural effects with those of morphine and Δ-9-tetrahydrocannabinol (THC). Sucrose-conditioned place preference test represent the effects of repeated drug intervention on the hedonic value of natural reward (Carlezon & Chartoff 2007; Donahue *et al.* 2014; Radwanska & Kaczmarek 2012).

MATERIALS AND METHODS

Animals

Thirty adult male Swiss albino mice (30–40 g) were obtained from and maintained at the Animal Research and Service Centre of Universiti Sains Malaysia (Health Campus) in Kelantan, Malaysia, where they were bred and kept as litter groups until shortly before the testing began. The mice were approximately 3 months old at the beginning of the experiment and had not been subjected to any prior testing. One week before being introduced to the IntelliCage®, the animals were housed six per cage, respective to their assigned intervention groups, in polypropylene cages with standard laboratory mouse feed and tap water *ad libitum*, under controlled environmental conditions (temperature 22 ± 2 °C;12/12-h light–dark schedule). Throughout habituation and experimentation, the gross behaviour of each mouse was observed. Mice showing any signs of aggressive behaviour were excluded from the analysis. All mice showed no signs of aggression (i.e. no attacks/fighting and biting) that may affect their social behaviour.

All animals were sacrificed by CO_2 euthanisation in a closed chamber 24 h at the end of this study. In all cases, the animals were maintained according to the specified duration of pre- and post-exposure. All procedures involving animal handling and behavioural experimentation were approved and strictly complied with the guidelines of the Animal Ethics Committee of Universiti Sains Malaysia [USM/Animal Ethics Approval/2011/(72)(332)].

Drugs and chemicals

Mitragynine was provided by Centre for Drug Research, Universiti Sains Malaysia. Fresh *M. speciosa* leaves were harvested from Perlis, Malaysia and authenticated and deposited at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia (Voucher No.: USM11074). Mitragynine, the active principal alkaloid of *M. speciosa*, was isolated by the method reported by Jamil *et al.* (2013). The dried mitragynine extract was sealed in a bottle and stored at 4 °C until use.

Morphine sulphate (Pharmaniaga, Malaysia) and Δ-9-THC (Lipomed AG, Switzerland) were used as reference drugs. Tween 20 (Bio-rad, USA) was used as a vehicle.

IntelliCage® apparatus

The IntelliCage® system (NewBehavior AG, Switzerland) is a computer-based fully automated behavioural testing apparatus used to analyse individual recording of long-term and multi-dimensional behavioural patterns of radio frequency identification (RFID)-tagged mice in a social-group environment. The IntelliCage® system is a standard plastic cage equipped with four triangular operant test chambers. Entry into each operant chamber is via a ring antenna which detects animal's unique ID tags and records their visits. The round apertures on the walls of each chamber provide free access to water bottles. Small motorised doors at the aperture can be closed to limit access to water. Animals can be trained to perform fixed or progressive ratio nose pokes at the door to allow access to water. Four small shelters are placed at the middle of the cage as a form of enrichment (Codita *et al.* 2010; Krackow *et al.* 2010; Mechan *et al.* 2009; Voikar *et al.* 2010).

Experimental protocol

All animals were subcutaneously (s.c.) implanted with sterile RFID microtransponders (TSE System GmbH, Germany) with unique ID codes using supplied disposable syringe and injector. After 48 h of implantation, all animals were checked for retention of microtransponders with a handheld electronic reader (TSE System GmbH, Germany) before being released into the IntelliCage®. Following introduction into the IntelliCage®, the mice were allowed free access to all home-cage corners for four days to measure their baseline exploratory behaviours prior to drug intervention. Subsequently, the mice were subjected to daily sensitisation for 28 days according to their assigned drug intervention (i.e. in the IntelliCage®, animals were housed in cohorts of six mice at once and all received the same drug and dosage). Concurrently, the IntelliCage® system measured the effects of different drug interventions throughout the sensitisation period (Fig. 1).

Drug treatment

All animals were sensitised with drug treatments in a volume of 1 ml/kg body weight for 28 consecutive days ($n = 6$ animals/group). Mitragynine was freshly dissolved in 20 percent (*v*/*v*) Tween 20 for intraperitoneal administration (i.p.), as previously described (Hazim *et al.* 2011; Idayu *et al.* 2011), in incremental dosage from 5 to 25 mg/kg, in increments of 5 mg/kg (day 1–3: 5 mg/kg; day 4–6: 10 mg/kg; day 7–9: 15 mg/kg; day 10–12: 20 mg/kg; day 13–28: 25 mg/kg). The selected dose of mitragynine has previously been shown to affect locomotor activities, cognition and memory in mice (Apryani *et al.* 2010). Morphine sulphate was freshly dissolved in physiological saline (0.9 percent NaCl). THC was first dissolved in ethanol then mixed with 0.9 percent NaCl. The selected dose of morphine sulphate (5 mg/kg, s.c.) and THC (2 mg/kg, i.p.) was based upon the basis of

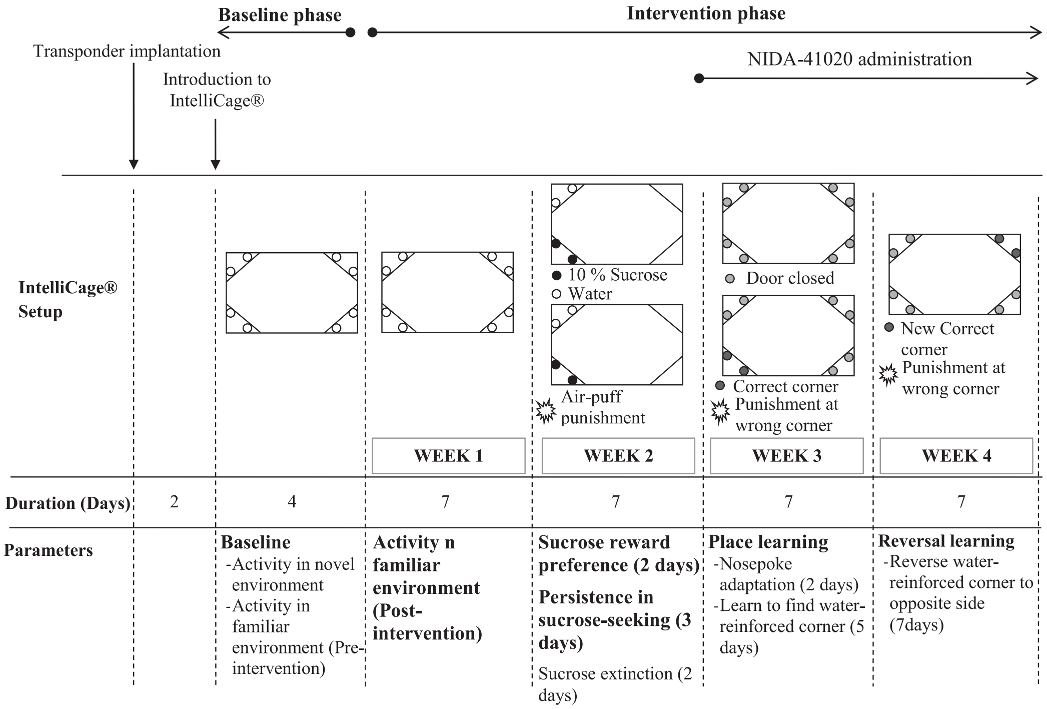
Baseline phase
Intervention phase
Transponder implantation
Introduction to IntelliCage®
NIDA-41020 administration
IntelliCage® Setup
● 10 % Sucrose
○ Water
Air-puff punishment
Door closed
Correct corner
Punishment at wrong corner
New Correct corner
Punishment at wrong corner
WEEK 1
WEEK 2
WEEK 3
WEEK 4
Duration (Days)
2
4
7
7
7
7
Parameters
Baseline
-Activity in novel environment
-Activity in familiar environment (Pre-intervention)
Activity n familiar environment (Post-intervention)
Sucrose reward preference (2 days)
Persistence in sucrose-seeking (3 days)
Sucrose extinction (2 days)
Place learning
-Nosepoke adaptation (2 days)
-Learn to find water-reinforced corner (5 days)
Reversal learning
-Reverse water-reinforced corner to opposite side (7days)

Figure 1 Summary of IntelliCage® experimental schedule

previous studies showing the development of morphine- (Abdel-Zaher *et al*. 2013) and THC- (Zuardi, Hallak, & Crippa 2012) tolerance and dependence in mice, respectively. Vehicle group received daily i.p. administration of 20 percent (*v*/*v*) Tween 20. One group did not receive any treatment. All mice were taken out once daily (in the morning) from the IntelliCage® system to receive their drug intervention accordingly. Mice were returned back to IntelliCage® immediately after intervention.

Behavioural parameters in the IntelliCage® system

Parameters measured were adapted with minor modifications from Radwanska & Kaczmarek (2012). All 30 animals responded well to the apparatus throughout experimentation; thus, all were included for analysis.

Exploratory activity and sensitization

Baseline phase (pre-intervention). Mice had free access to all areas of the cage for four days without receiving any drug treatment. All water-access doors were opened. Exploratory activity was measured as the number of visits performed during the first hour in the IntelliCage® (exploratory activities in novel environment) and the following three consecutive days (baseline exploratory activity in familiar environment). The latter data were determined for comparison with the mice activities upon introduction to drug treatment.

Activity during drug treatment (post-intervention):. IntelliCage® setup during this phase was identical to baseline phase. All mice received their drug intervention once daily and were returned back to IntelliCage® immediately after intervention. Data collected during this phase comprised of the number of visits per day for seven consecutive days following drug intervention. As established in previous studies, the mean number of corner visits was used as a proxy for general exploratory activity (Galsworthy *et al*. 2005; Radwanska & Kaczmarek 2012; Robinson & Riedel 2014) and compared with the activities prior to drug treatment.

Sucrose reward preference

In order to measure preference of natural rewards during chronic drug treatment, sucrose preference was measured for two days. Two IntelliCage® corners were active and freely accessible to mice. In one active corner, the mice had access to normal tap water. In the other active corner a 10 percent sucrose solution was available. The doors of two remaining corners were closed throughout this phase. The number of licks at the sucrose bottle was measured in relation to licks at the water bottle and a percent preference for sucrose calculated from Day 8 to Day 9 of drug intervention.

Persistence in sucrose preference

The persistence in sucrose reward-seeking was measured by calculating the reduction in sucrose consumption when it was associated with an air-puff punishment. This phase lasted three days where the mice had free access to two active corners with normal tap water and 10 percent sucrose, respectively. Any licks performed at the sucrose-reward corner resulted in an air-puff as a form of punishment to the mice. The remaining two days after sucrose persistence study served as sucrose extinction phase in order to prepare the mice for subsequent phase. The percent of sucrose preference with versus without punishment was determined.

Place learning and reversal

Nose poke adaptation. This phase lasted two days where the water-access doors were closed at all corners. Upon a nosepoke, the respective door was opened for 5 s for access to water. The least preferred corners of each individual mouse were determined for programming subsequent place learning phase. *Place learning:* All doors remained closed during a 5-day place learning phase. For each animal, access to water was restricted to one correct water-rewarded corner (individually least preferred corner during nosepokes adaptation). Three successive nosepokes at an individually correct corner opened the respective door for 5 s. The percent preference for the rewarded corner was determined. *Place learning reversal:* The same procedure as for acquisition of place learning was used, except that now the water-rewarded corner was changed to the diagonal opposite. This phase lasted for seven days. Thereby, the percent preference for the newly rewarded corner was determined.

Statistical analyses

The data from IntelliCage® system were analysed using IntelliCage® Analyzer software (IntelliCage® Plus 2.4, NewBehavior AG, Switzerland) and SPSS 22.0 (IBM Corp., USA). Data were reported as mean ± SEM. Paired samples *t*-test was used for comparison within a treatment group [baseline exploratory activity (pre) versus sensitisation of exploratory activity (post)]. Comparison between groups was performed using one-way and two-way repeated measures of ANOVA, followed by Tukey's HSD post hoc tests for multiple comparisons. For all analyses, $p < 0.05$ was considered to be statistically significant.

RESULTS

Mitragynine leads to a sensitization of exploratory activity

Baseline exploratory activity

Using the IntelliCage® platform, mice novelty-induced exploration was measured by the mean number of corner visits during the first hour in the novel IntelliCage®, while daily baseline activities were measured by the mean number of corner visits during three consecutive days in familiar IntelliCage® environment. No significant difference was displayed in the number of corner visits performed in novel environment ($p > 0.05$) and familiar environment ($p > 0.05$) during pre-drug baseline phase.

Sensitization of exploratory activity

The effects of seven day drug treatment on locomotor sensitization are shown in Fig. 2. Untreated and vehicle groups did not show any significant difference between baseline and post-intervention exploratory activities in the familiar IntelliCage® environment ($p > 0.05$). Morphine- and mitragynine-treated groups performed significantly more corner visits following drug intervention (morphine: $t(5) = -11.44$, $p < 0.001$; mitragynine: $t(5) = -16.28$, $p < 0.001$). In contrast, the THC-treated group performed significantly less visits to IntelliCage® corners after treatment ($t(5) = 9.74$, $p < 0.001$).

The effect of drug intervention on sucrose reward preference

When exposed to a two-day period of two bottle choice test, regardless of the intervention received, all mice showed overall strong preference for sucrose over water ($F_{1,\ 30} = 1365.14$, $p < 0.001$; Fig. 3A). A significant increase in lick preference for the sucrose-corner as opposed to pre-sucrose was found in all groups ($F_{1,30} = 1365.14$, $p < 0.001$). There was also a statistically significance effect observed between drug intervention groups ($F_{5,30} = 46.32$, $p < 0.001$). Post hoc tests indicated that all three drug-sensitised groups elicited a stronger preference (i.e. increasing percent of reward licks at sucrose-associated corner) for sucrose reward (mitragynine: 54 ± 1.3 percent; morphine: 57 ± 0.5 percent, 54 ± 0.7 percent increment, $p < 0.001$) in comparison to untreated and vehicle-control groups (38 ± 2.5 percent and 42 ± 3.0 percent increment, respectively, $p < 0.01$). Interestingly, the mitragynine-sensitised group showed similar preference for sucrose reward as morphine and THC-sensitised groups, which may suggest comparable enhancement in natural reward seeking after chronic treatment.

Mitragynine enhances punishment resistance of sucrose-seeking

A repeated measures ANOVA confirmed a significant reduction in sucrose consumption when the reward corner was paired with an air-puff punishment, irrespective of intervention groups ($F_{1,30} = 652.43$, $p < 0.001$; Fig. 3B). The persistence in sucrose-seeking was found to significantly differ between intervention groups ($F_{5,30} = 155.57$, $p < 0.001$). Post-hoc comparisons indicated that drug sensitised-mice were significantly more resistance to punishment in their sucrose seeking. During punishment sucrose seeking was maintained to a level of 62 ± 1.4 percent after morphine, 65 ± 1.4 percent after mitragynine and 78 ± 1.4 percent after THC ($p > 0.05$, versus without punishment). In the control groups sucrose seeking was maintained at a level of 20 ± 1.4 percent for untreated, and 26 ± 1.4 percent after vehicle treatment ($p < 0.005$, versus without punishment). These findings suggest that mitragynine enhances punishment resistance of sucrose seeking behaviour.

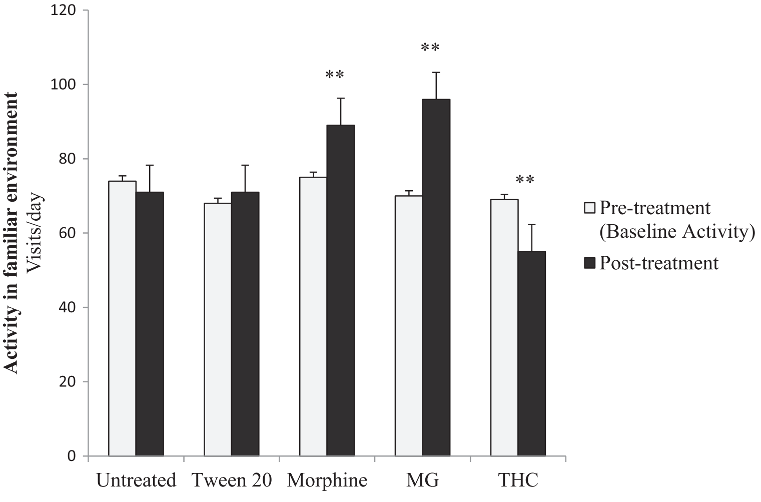

Figure 2 Effects of drug intervention to daily activity in familiar IntelliCage® environment. Data are shown as the mean number of visits to all corners per test group during baseline phase (prior to drug intervention) and seven days of post-treatment phase (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; paired t-test, $n = 6$/group, MG-mitragynine; THC = Δ-9-Tetrahydrocannabinol)

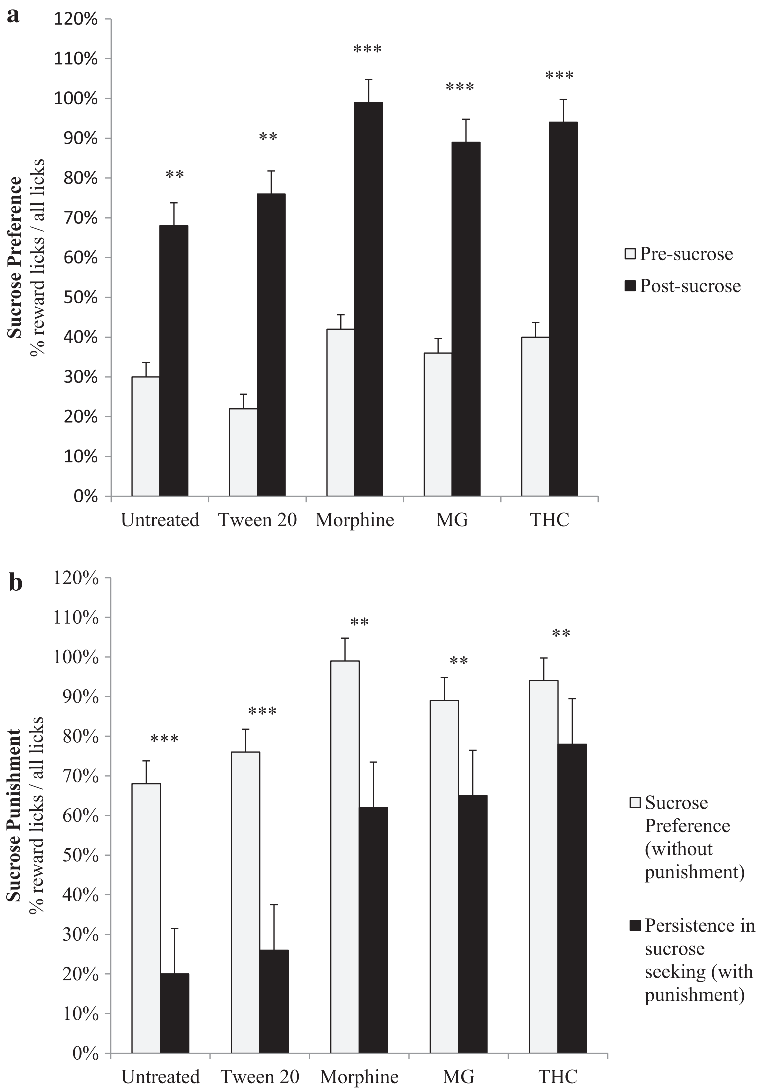

Figure 3 Effects of drug intervention on preference for sucrose reward. A.) Data are shown as the percentage of licks at the reward (10 percent sucrose) corner to all licks measured during the two-day sucrose preference phase. B.) Effects of drug intervention on persistence of sucrose-seeking. Data are shown as the percentage of licks at the reward (10 percent sucrose) corner, when paired with air-puff punishment, to all licks measured during the three-day phase (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$;repeated measures ANOVA, $n = 6$/group, MG-mitragynine; THC = Δ-9-Tetrahydrocannabinol)

Mitragynine impairs place learning and its reversal

Place learning

Place learning was significantly impaired after chronic treatment with mitragynine, morphine or THC (Fig. 4A). A two-way repeated measures ANOVA yielded a significant effect for factor treatment ($F_{5,30} = 11.9$, $p < 0.05$), day ($F_{5,30} = 3.31$, $p < 0.05$), and a significant treatment × day interaction ($F_{5,30} = 34.3$, $p < 0.001$). Untreated and vehicle treated groups showed a significant increase in the preference for the correct corner over days of testing (untreated: $F_{1,5} = 20.74$, $p < 0.05$; vehicle: $F_{1,5} = 20.74$, $p < 0.05$), with no significant difference between the two groups ($p > 0.05$). No such improvement in place learning by days was observed in the three sensitised groups (all: $p > 0.05$). Thereby, the deficit in place learning in the mitragynine-sensitised mice did not differ from morphine- or THC-treated animals ($p > 0.05$).

Place learning reversal

Also the reversal of a rewarded place was learned less well after chronic treatment with mitragynine, morphine or THC (Fig. 4B). A repeated measures ANOVA yielded a significant effect for factor treatment ($F_{5,30} = 11.9$, $p < 0.05$), day ($F_{5,30} = 3.31$, $p < 0.05$), and a significant treatment × day interaction ($F_{5,30} = 38.9.3$, $p < 0.001$). Untreated and vehicle treated groups showed a significant increase in the preference for the new correct corner over days of testing (untreated: $F_{1,5} = 5.74$, $p < 0.05$; vehicle: $F_{1,5} = 12.32$, $p < 0.05$), with no significant difference between the two groups ($p > 0.05$). Consistent with the results from place learning phase, all drug-sensitised groups failed to acquire the newly placed water-reinforced corner (all: $p > 0.05$). Repeated measures ANOVA also showed that all mice showed no significant difference in the number of lick frequency during place learning phase ($F_{1,\ 30} = 13.65$, $p > 0.05$) and reversal learning phase ($F_{1,\ 30} = 22.14$, $p > 0.05$).

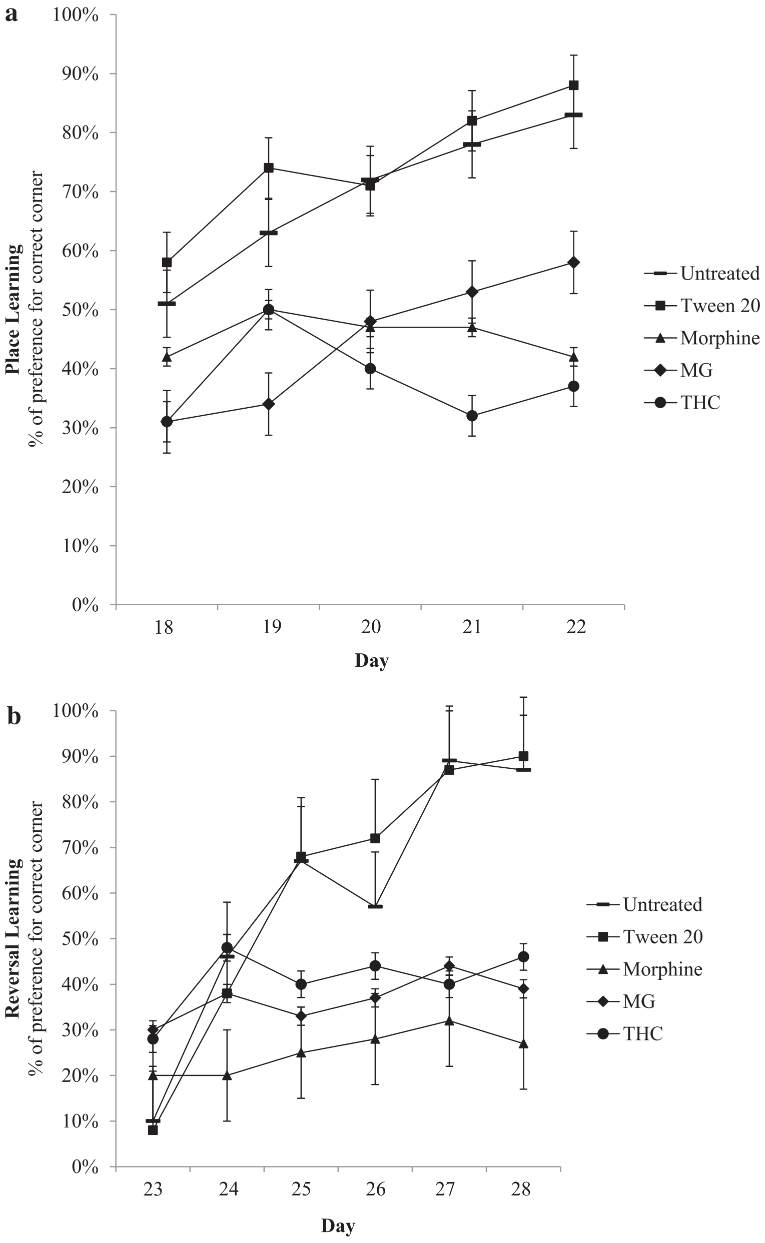

Figure 4 Effects of drug intervention on place learning and reversal learning. Data are shown as the percentage of preference for the correct (water-reinforced) corner, A.) during five days of place learning phase, as well as, B.) during six days of a reversal learning phase. Data revealed highly significant learning and reversal learning in untreated and vehicle treated groups. All three drug-sensitised groups (morphine, MG-mitragynine and THC-Δ9-tetrahydrocannabinol) failed to acquire place learning and learn the reversal. (two-way repeated measures ANOVA, *n* = 6/group, MG-mitragynine; THC = Δ-9-Tetrahydrocannabinol)

Note that IntelliCage® does not measure the amount of liquid intake. Thus, the number of licks was used as a proxy for amount of mice total water intake.

DISCUSSION

In this study we presented the first longitudinal behavioural assessment of group-housed mice, using the automated IntelliCage® behavioural system, sensitised with mitragynine, morphine or THC. A fully automated social group instrument such as IntelliCage® is intended to reduce human handling and social environmental discrepancies so as to minimise interferences of animal's behavioural activities. Our study revealed that mitragynine significantly enhanced exploratory activity in mice, shown by the increased number of IntelliCage® corner visits. Evidently, acute mitragynine administration was reported to induce significant increase of arm explorations in Y-maze test (Hazim *et al.* 2011) and elevated plus maze test, and central zone explorations in open-field test (Hazim *et al.* 2014). Studies suggested the occurrence of locomotor sensitisation in response to repetitive exposure to abused drugs, resulting in progressive amplification of behavioural and locomotor activity (Justinova, Palilio, & Goldberg 2009; Koob 2011). We found that in the IntelliCage® social setting that also chronic morphine treatment induced a sensitization of exploratory behaviour. Given that mitragynine acts as an opioid agonist, among other as yet undetermined receptor affinities, one may speculate that it enhances exploration by acting on opioid receptors in mesolimbic and nigrostriatal dopaminergic systems (Ford, Mark, & Williams 2006; Li *et al.* 2010). In contrast, reduced exploratory activity was observed after chronic THC treatment, consistent with the result from Dow-Edwards and Izenwasser (Dow-Edwards & Izenwasser 2012). This was suggested to result from the development of cannabinoid tolerance, causing rapid down-regulation of endocannabinoid receptor binding sites in mesocorticolimbic circuitry and cerebellum (Justinova *et al.* 2009; Martin 2005). This neural adaptation seems to be responsible for the development of cannabinoid tolerance, causing subsequent diminished locomotion.

Mice chronically treated with mitragynine, morphine, or THC showed significantly higher preference for a sucrose-associated corner compared with untreated and vehicle-treated groups. This suggests an increase in the sucrose incentive value in drug sensitised mice. It was suggested that sweet preference may serve as a predictor for substance abuse due to similar overlapping mechanism related to the rewarding effects of addictive drugs and sucrose (Levine, Kotz, & Gosnell 2003). Gosnell (2000)reported that rats with high ingestion of sucrose show higher frequency of cocaine self-administration. Also, Jarrett, Limebeer, & Parker (2005) found that THC enhanced sucrose palatability, an effect which was reversed by CB_1 cannabinoid receptor antagonist, SR141716. Sudden air-puff stimuli have been shown to significantly increase mice heart rate suggesting an aversive emotional state (Liu *et al.*, 2014). Evidently, all mice showed a significant decline in the frequency of licks at sucrose-associated corner when it was paired with air-puff punishment, representing a decrement in the persistency of sucrose-seeking. Mice that received a chronic treatment with mitragynine, morphine or THC showed significantly higher persistence in sucrose seeking despite air-puff punishment, compared with untreated and vehicle control groups. Possible increase ability in resisting air-puff punishment as well as more motivated in securing pleasurable effects in these treated mice may account for this finding. Neuroadaptation in the mesocorticolimbic system, particularly in the amygdala which governs fear-conditioning, may result in the addicted mice ability to resist punishment. Also, upon repeated exposure to addictive drugs, extinction of long-lasting aversive memory may occur, which coincides with reports of learning and memory impairment associated with mitragynine (Apryani *et al.* 2010; Yusoff *et al.* 2014), morphine (Lu *et al.* 2010) and THC exposure (Justinova *et al.* 2009), which may reflect the persistent pursuits of drugs observed in human addicts. Contemporary addiction theories implicate the cognitive control over drug intake, value-based decision making and impulse control are synergistically mediated by the frontal cortex (Limpens *et al.* 2014). Taken together with the present findings, in the context of human behaviour, it is possible that mitragynine-induced impairment at these regions contributes to ineffective decision making in complex situations involving positive/negative consequences, eventually leading to compulsive aspects of kratom use.

Place learning and reversal learning paradigms were designed to evaluate the effects of drug interventions mice's spatial learning ability. From this study, the finding that chronic mitragynine abolished place learning for a water-reinforced corner supports an emerging literature that implicates mitragynine-induced cognitive impairment (Apryani *et al.* 2010; Senik *et al.* 2012; Yusoff *et al.* 2014), although the exact mechanisms remain elusive. Apryani *et al.* (2010) demonstrated that chronic administration (28 days) with 5 to 15 mg/kg of mitragynine impaired working memory processes in mice. An independent dose of 20, 40 and 80 mg/kg of mitragynine significantly increases horizontal and vertical exploration of Swiss albino mice in novel Y-maze environment. However, the same study indicated no significant effect on short-term memory processes and motor coordination using Y-maze and rota-rod test, respectively (Hazim *et al.* 2011). On the contrary, a single administration of high dosage kratom standardised methanol extract significantly improved learning acquisition, but impaired long-term memory consolidation, of rats in passive and active avoidance tests (Senik *et al.* 2012). A more recent study described that acute mitragynine impaired passive avoidance learning, memory consolidation and retrieval, while chronic mitragynine led to impairment of passive avoidance and object recognition learning in rodents (Yusoff *et al.* 2014).

Place learning is generally considered to be a hippocampal-dependent task and *M. speciosa* methanol extract has been demonstrated to exert short-term potentiation CA1 region of rat hippocampal slices (Senik *et al.* 2013). Our study also reveals morphine- and THC-sensitised mice failed to find and learn the location of water-reinforced corner. This is corroborated by a recent study on morphine-dependent mice which reported significant reduction of spine density in hippocampal CA1 region (Yang *et al.* 2013), while chronic THC may impair learning and memory by inhibiting GABA release in the hippocampus (Laaris, Good, & Lupica 2010). Therefore, neuroplasticity in the brain regions governing place learning and memory, following chronic exposure to addictive drugs, may account for spatial learning and reversal learning impairment in our study. Meanwhile, recent cross-sectional studies reported the development of dependency, tolerance, craving and withdrawal symptoms following abstinence among medium-term and long-term kratom users in Malaysia and Thailand

(Ahmad & Aziz 2012; Saingam *et al.* 2013; Singh *et al.* 2014; Vicknasingam *et al.* 2010). These results implied that chronic learning and memory deficits could be a direct effect of prolonged kratom use, consequently suggesting its putative influence on the memory system. However, the underlying mechanisms call for further evaluation.

Our data demonstrated that chronic administration of mitragynine significantly enhanced exploratory activity in a similar way as chronic morphine treatment did. Mitragynine-sensitised mice showed a significantly higher preference for natural reward (10 percent sucrose) and more persistence in reward-seeking during parallel aversive consequences (air-puff punishment). Also in that respect, mitragynine effects resembled close that of morphine. Furthermore, we could show that spatial learning and memory process were impaired during chronic mitragynine treatment. Also the reversal of the rewarded spatial location was learned less well than in controls. Again, the observed learning deficits were similar to those induced by chronic morphine or THC treatment. The induction of morphine- and cannabinoid-like behavioural alterations may suggest that mitragynine could possibly act through the same targets in the brain as morphine and/or THC. Further investigations are warranted to elucidate the underlying neurological adaptations which complement these behavioural changes. At the same time, the IntelliCage® test should be further refined and extended by designing a more complex conditioning tasks and parameters for rodent in addiction studies.

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AUTHOR CONTRIBUTION

NIWI and NJ performed the experiments and conducted data acquisitions. All authors made substantial contributions to the conception, analysis and interpretation of data; drafting the article and revising it critically for important intellectual content; and approved the final manuscript.

References

Abdel-Zaher AO, Mostafa MG, Farghaly HS, Hamdy MM, Abdel-Hady RH (2013) Role of oxidative stress and inducible nitric oxide synthase in morphine-induced tolerance and dependence in mice. Effect of alpha-lipoic acid. Behav Brain Res 247:17–26.

Ahmad K, Aziz Z (2012) *Mitragyna speciosa* use in the northern states of Malaysia: a cross-sectional study. J Ethnopharmacol 141:446–450.

Arndt T, Claussen U, Güssregen B, Schröfel S, Stürzer B, Werle A, Wolf G (2011) Kratom alkaloids and O-desmethyltramadol in urine of a “Krypton” herbal mixture consumer.. Forensic Science International 208:47–52.

Apryani E, Taufik Hidayat M, Moklas M, Fakurazi S, Farah Idayu N (2010) Effects of mitragynine from *Mitragyna speciosa* Korth leaves on working memory. J Ethnopharmacol 129:357–360.

Carlezon WA, Chartoff EH (2007) Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Nat Protocols 2:2987–2995.

Codita A, Gumucio A, Lannfelt L, Gellerfors P, Winblad B, Mohammed AH, Nilsson LN (2010) Impaired behavior of female tg-ArcSwe APP mice in the IntelliCage: a longitudinal study. Behav Brain Res 215:83–94.

Cornara L, Borghesi B, Canali C, Andrenacci M, Basso M, Federici S, Labra M (2013) Smart drugs: green shuttle or real drug?. International Journal of Legal Medicine 127:1109–1123.

Donahue RJ, Muschamp JW, Russo SJ, Nestler EJ, Carlezon WA Jr (2014) Effects of striatal ΔFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice. Biol Psychiatry 76:550–558.

Dow-Edwards D, Izenwasser S (2012) Pretreatment with Δ9-tetrahydrocannabinol (THC) increases cocaine-stimulated activity in adolescent but not adult male rats. Pharmacol Biochem Behav 100:587–591.

Dresen S, Ferreirós N, Pütz M, Westphal F, Zimmermann R, Auwärter V (2010) Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. Journal of Mass Spectrometry 45:1186–1194.

Edwards S, Koob GF (2013) Escalation of drug self-administration as a hallmark of persistent addiction liability. Behav Pharmacol 24:356–362.

Ford CP, Mark GP, Williams JT (2006) Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. J Neurosci 26:2788–2797.

Forrester MB (2013) Kratom exposures reported to Texas poison centers. Journal of Addictive Diseases 32:396–400.

Galsworthy MJ, Amrein I, Kuptsov PA, Poletaeva II, Zinn P, Rau A, Vyssotski A, Lipp H-P (2005) A comparison of wild-caught wood mice and bank voles in the Intellicage: assessing exploration, daily activity patterns and place learning paradigms. Behav Brain Res 157:211–217.

Goldberg R (2014) Drugs Across the Spectrum, 7th edn. Wadsworth Cengage Learning: CA.

Gosnell BA (2000) Sucrose intake predicts rate of acquisition of cocaine self-administration. Psychopharmacology (Berl) 149:286–292.

Hassan Z, Muzaimi M, Navaratnam V, Yusoff NHM, Suhaimi FW, Vadivelu R, Vicknasingam BK, Amato D, von Hörsten S, Wan Ismail NI, Jayabalan N, Hazim AI, Mansor SM, Müller CP (2013) From Kratom to mitragynine and its derivatives: physiological and behavioural effects related to use, abuse, and addiction. Neurosci Biobehav Rev 37:138–151.

Hazim AI, Mustapha M, Mansor SM (2011) The effects on motor behaviour and short-term memory tasks in mice following an acute administration of *Mitragyna speciosa* alkaloid extract and mitragynine. J Med Plants Res 5:5810–5817.

Hazim AI, Ramanathan S, Parthasarathy S, Muzaimi M, Mansor SM (2014) Anxiolytic-like effects of mitragynine in the open-field and elevated plus-maze tests in rats. J Physiol Sci 64:161–169.

Idayu NF, Hidayat MT, Moklas M, Sharida F, Raudzah AN, Shamima A, Apryani E (2011) Antidepressant-like effect of mitragynine isolated from *Mitragyna speciosa* Korth in mice model of depression. Phytomedicine 18:402–407.

Jamil MFA, Subki MFM, Lan TM, Majid MIA, Adenan MI (2013) The effect of mitragynine on cAMP formation and mRNA expression of mu-opioid receptors mediated by chronic morphine treatment in SK–N–SH neuroblastoma cell. J Ethnopharmacol 148:135–143.

Jansen K, Prast CJ (1988) Ethnopharmacology of kratom and the Mitragyna alkaloids. J Ethnopharmacol 23:115.

Jarrett MM, Limebeer CL, Parker LA (2005) Effect of Δ 9-tetrahydrocannabinol on sucrose palatability as measured by the taste reactivity test. Physiol Behav 86:475–479.

Justinova Z, Palilio LV, Goldberg SR (2009) Drug addiction. In: Kendell D, Alexander S eds. Behavioural Neurobiology of the Endocannabinoid System, pp 309–346. Springer: UK.

Koob GF (2011) Neurobiology of addiction. FOCUS: J Lifelong Learn Psychiatry 9:55–65.

Krackow S, Vannoni E, Codita A, Mohammed A, Cirulli F, Branchi I, Alleva E, Reichelt A, Willuweit A, Voikar V (2010) Consistent behavioral phenotype differences between inbred mouse strains in the IntelliCage. Genes Brain Behav 9:722–731.

Laaris N, Good CH, Lupica CR (2010) Δ9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. Neuropharmacology 59:121–127.

Levine AS, Kotz CM, Gosnell BA (2003) Sugars: hedonic aspects, neuroregulation, and energy balance. Am J Clin Nutr 78:834S–842S.

Li T, Hou Y, C-x Y, Chen T, Zhao Y, S-b L (2010) Dopamine D3 receptor knock-out mice display deficits in locomotor sensitization after chronic morphine administration. Neurosci Lett 485:256–260.

Limpens JHW, Damsteegt R, Broekhoven MH, Voorn P, Vanderschuren LJMJ (2014) Pharmacological inactivation of the prelimbic cortex emulates compulsive reward seeking in rats. Brain Res 1628:210–218.

Liu J, Wei W, Kuang H, Tsien JZ, Zhao F (2014) Heart rate and heart rate variability assessment identifies individual differences in fear response magnitudes to earthquake, free fall, and air puff in mice. PloS one 9:e93270.

Lu G, Zhou Q-X, Kang S, Li Q-L, Zhao L-C, Chen J-D, Sun J-F, Cao J, Wang Y-J, Chen J (2010) Chronic morphine treatment impaired hippocampal long-term potentiation and spatial memory via accumulation of extracellular adenosine acting on adenosine A1 receptors. J Neurosci 30:5058–5070.

Martin BR (2005) Role of lipids and lipid signaling in the development of cannabinoid tolerance. Life Sci 77:1543–1558.

Mechan AO, Wyss A, Rieger H, Mohajeri MH (2009) A comparison of learning and memory characteristics of young and middle-aged wild-type mice in the IntelliCage. J Neurosci Methods 180:43–51.

Müller CP, Homberg JR (2015) The role of serotonin in drug use and addiction. Behav Brain Res 277:146–192.

NADA (2013) Laporan dadah Disember 2013, pp 1–39. Malaysia: National Anti-Drugs Agency.

Radwanska K, Kaczmarek L (2012) Characterization of an alcohol addiction-prone phenotype in mice. Addict Biol 17:601–612.

Robinson L, Riedel G (2014) Comparison of automated home-cage monitoring systems: emphasis on feeding behaviour, activity and spatial learning following pharmacological interventions. J Neurosci Methods 234:13–25.

Sabetghadam A, Ramanathan S, Sasidharan S, Mansor SM (2013) Subchronic exposure to mitragynine, the principal alkaloid of *Mitragyna speciosa*, in rats. J Ethnopharmacol 146:815–823.

Saingam D, Assanangkornchai S, Geater AF, Balthip Q (2013) Pattern and consequences of krathom (*Mitragyna speciosa* Korth.) use among male villagers in southern Thailand: a qualitative study. Int J Drug Policy 24:351–358.

Sakaran R, Othman F, Jantan I, Thent ZC, Das S (2014) An insight into the effect of *Mitragyna speciosa* Korth extract on various systems of the body. Global J Pharmacol 8:340–346.

Senik MH, Mansor SM, Rammes G, Tharakan J, Abdullah J (2013) *Mitragyna speciosa* Korth standardized methanol extract induced short-term potentiation of CA1 subfield in rat hippocampal slices. J Med Plants Res 6:1234–1243.

Senik MH, Mansor SM, Tharakan JKJ, Abdullah JM (2012) Effect of acute administration of *Mitragyna speciosa* Korth. standardized methanol extract in animal model of learning and memory. J Med Plants Res 6:1007–1014.

Shamima AR, Fakurazi S, Hidayat MT, Hairuszah I, Moklas MAM, Arulselvan P (2012) Antinociceptive action of isolated mitragynine from *Mitragyna speciosa* through activation of opioid receptor system. Int J Mol Sci 13:11427–11442.

Singh D, Müller CP, Vicknasingam BK (2014) Kratom (*Mitragyna speciosa*) dependence, withdrawal symptoms and craving in regular users. Drug Alcohol Depend 139:132–137.

Suwanlert S (1975) A study of kratom eaters in Thailand. Bulletin on Narcotics.

Takayama H (2004) Chemistry and pharmacology of analgesic indole alkaloids from the rubiaceous plant, *Mitragyna speciosa*. Chem Pharm Bull 52:916–928.

Thongpradichote S, Matsumoto K, Tohda M, Takayama H, Aimi N, Sakai S-I, Watanabe H (1998) Identification of opioid receptor subtypes in antinociceptive actions of supraspinally-administered mitragynine in mice. Life Sci 62:1371–1378.

Vicknasingam B, Narayanan S, Beng GT, Mansor SM (2010) The informal use of ketum (*Mitragyna speciosa*) for opioid withdrawal in the northern states of peninsular Malaysia and implications for drug substitution therapy. Int J Drug Policy 21:283–288.

Voikar V, Colacicco G, Gruber O, Vannoni E, Lipp HP, Wolfer DP (2010) Conditioned response suppression in the IntelliCage: assessment of mouse strain differences and effects of hippocampal and striatal lesions on acquisition and retention of memory. Behav Brain Res 213:304–312.

Yang S, Wen D, Dong M, Li D, Sun D, Ma C, Cong B (2013) Effects of cholecystokinin-8 on morphine-induced spatial reference memory impairment in mice. Behav Brain Res 256:346–353.

Yusoff NH, Suhaimi FW, Vadivelu RK, Hassan Z, Rümler A, Rotter A, Amato D, Dringenberg HC, Mansor SM, Navaratnam V, Müller CP (2014) Abuse potential and adverse cognitive effects of mitragynine (kratom). Addict Biol 21:98–110.

Zuardi AW, Hallak JEC, Crippa JAS (2012) Interaction between cannabidiol (CBD) andΔ9-tetrahydrocannabinol (THC): influence of administration interval and dose ratio between the cannabinoids. Psychopharmacology (Berl) 219:247–249.